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N. WOODSON
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
ZHUANGWU	LI	Clarksville, Maryland

[X] Additional inventors are being named on the 1 separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

Methods For Detecting Telomerase Activity

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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OR

[X] Firm or Individual Name	ZHUANGWU LI				
Address	6054 Signal Flame Ct				
Address					
City	Clarksville	State	MD	ZIP	21029
Country	USA	Telephone	4435350565	Fax	443 535-0565

ENCLOSED APPLICATION PARTS (check all that apply)

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METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.
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Respectfully submitted,
SIGNATURE Zhuangwu Li
Date 11/12/02
TYPED or PRINTED NAME ZHUANGWU LI
REGISTRATION NO. (if appropriate)
TELEPHONE 443 535-0565
Docket Number:

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PROVISIONAL APPLICATION COVER SHEET
Additional Page

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INVENTOR(S)/APPLICANT(S)

Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
Jun	Bao	17064 E. Colima Rd, #259 Hacienda Heights, CA 91745 Phone: 626.810-3467

Number 2 of 2

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METHODS FOR DETECTING TELOMERASE ACTIVITY

TECHNICAL FIELD

The present invention generally relates to medical diagnostic and prognostic technology. In particular, the present invention relates to a method for the detection of telomerase activity.

BACKGROUND

Telomerase is an enzyme that synthesizes telomeres on chromosome ends. Telomeres are DNA sequences found at the ends of eukaryotic chromosomes that maintain the fidelity of genetic information during replication. Under normal circumstances, telomeres become shorter and shorter with each cycle of cell division. A sufficiently short telomere is believed to signal the cells to stop dividing.

Telomerase belongs to a class of enzymes known as reverse transcriptases that use RNA as a template for creating DNA. Telomerase contains both RNA and protein components. The RNA portion of the enzyme binds to the DNA in the telomere while the protein component lures DNA subunits into the region and attaches them to the end of the chromosome. In the case of eukaryotic organisms, telomerases are composed of an accumulation of repeated defined nucleotide sequences (repeats), which for example contain the sequence TTAGGG in humans.

Telomerase activity is expressed in most human tumor tissues but not in normal tissues except those of the germline (testes/ovaries). Stem cells of renewing tissues express very low levels of telomerase. Telomerase activity is occasionally detected in tissues adjacent to tumors possibly reflecting the presence of occult micrometastases.

It has been suggested that telomerase is responsible for the unchecked growth of human cancer cells. Unlike normal cells, in cancer cells telomerase appears to grant the cell immortality by maintaining telomere length so that the cell never receives a signal to stop dividing.. The telomerase enzyme is an ideal target for chemotherapy because this enzyme is active in about 90 percent of human tumors, but inactive in most normal cells. Pharmaceutical companies have screened thousands of compounds to find agents capable of blocking telomerase.

A number of methods have been developed to measure telomerase activity. Most of them are based on the *in vitro* detection of the enzyme activity. Briefly, a synthetic oligonucleotide derived from the telomere sequence is used as a primer. This primer is elongated by the telomerase in a test sample; the product of synthesis is then amplified and quantified. The methods have been described in detail in, for example, US Pat. No, 5,891,639 to Harley et al. and US Pat. No. 6,221,584 to Emrich et al.

DESCRIPTION OF THE INVENTION

The present invention discloses a method to determine telomerase activity in a sample using primer extension followed with real time PCR quantification. The method provides a rapid and accurate measurement for telomerase activity in a biological sample. The present invention also concerns a suitable reagent kit for carrying out the method and for diagnoses of telomerase-related diseases.

In one embodiment, the present invention provides a method for detecting and quantifying telomerase activity in a biological sample. The method comprising the steps of: (a) adding the biological sample to a reaction mixture comprising a first primer and nucleoside triphosphates; (b) incubating the reaction mixture under conditions suitable for a

telomerase to produce an extension product from the first primer; and (c) quantifying the extension product using real-time PCR analysis.

The biological sample is preferably a cell extract, in particular an extract from human cells. The cell extract may be produced by repeated thawing/freezing of cells, or by lysing cells in a lysis buffer containing a non-ionic or/and zwitterionic detergent. The first primer is preferably an oligodeoxyribonucleotide suitable as a telomerase substrate. The length of the primer is preferably 10-50 and particularly preferably 12-30 nucleotides. The reaction mixture in step (a) may contain unlabelled nucleoside triphosphates or non-radioactive labeled nucleoside triphosphates. Preferably, the reaction mixture contains only unlabelled nucleoside triphosphates.

The extension product of the step (b) may be subjected to an additional template-independent elongation. This elongation is preferably achieved by means of an enzymatic reaction e.g. by attaching nucleotides e.g. by a polyadenylation using terminal transferase or by ligation of short DNA fragments by means of DNA ligase.

The extension product (with or without additional elongation) is quantified by the real time quantitative polymerase chain reaction method (RTQ-PCR). The PCR amplification is typically achieved by adding a suitable enzyme that can polymerize nucleic acids e.g. a nucleic acid polymerase or a nucleic acid ligase. It is preferable to use a thermostable enzyme and to carry out the amplification in several cycles. The type of amplification step is not critical for the method according to the invention. Two primers are preferably used for the amplification whereby one of the primers is the first primer in step (a) and a suitable complementary primer can be used as the second primer. The amplification product is formed by enzymatic catalysis e.g. by a template-dependent DNA polymerase by attaching nucleotides to the first and second primer. The second primer can preferably hybridize with the telomere repeat sequence. If an additional elongation e.g. by a terminal transferase is carried out after the extension, the second primer

can be complementary to the sequence section that is attached by elongation to the extension product.

Alternatively the amplification can be achieved by other methods known to a person skilled in the art. Thus the reaction can also be catalyzed by a template-dependent DNA ligase in which case the amplification product is formed by attaching an oligodeoxyribonucleotide to the primer by means of the DNA ligase. The DNA ligase is preferably a thermostable DNA ligase and such a method is particularly preferably carried out by means of the ligase chain reaction (LCR) technique.

The quantitative detection of the amplification product is achieved in a well-known manner by means of the labeling groups contained in the amplification product or via the labeling probes bound to the amplification product. The detection is preferably carried out using non-radioactive labeling agents in the RTQ-PCR. The non-radioactive labeling agents are preferably not introduced into the extension product until the quantification step (c). This can be achieved by using non-radioactively labeled CTP (not a component of the telomere repeat sequences) in the case that all the reaction steps are performed as a "one-pot reaction" without compartmentation. Alternatively, the non-radioactive labeling agents can be contacted with the reaction mixture at a later time. This can be achieved by compartmentation in which the non-radioactively labeling agents are separated during the extension step (b) from the reaction mixture by a removable barrier e.g. a wax layer that is meltable at higher temperatures. Another option is to add the reactants sequentially.

The non-radioactively labeling agents include DNA-binding dyes, TaqMan probes and molecular beacons. An example of DNA-binding dyes is SYBR Green, which binds to double-stranded DNA and emits light upon excitation. The TaqMan probes are oligonucleotides that contain a fluorescent dye, typically on the 5' base, and a quenching dye, typically located on the 3' base. When irradiated, the excited fluorescent dye transfers energy to the nearby quenching dye molecule rather than fluorescing, resulting in a

nonfluorescent substrate. This process is designated as fluorescence resonance energy transfer or FRET. TaqMan probes are designed to hybridize to an internal region of a PCR product. During PCR, when the polymerase replicates a template on which a TaqMan probe is bound, the 5 exonuclease activity of the polymerase cleaves the probe. This separates the fluorescent and quenching dyes and FRET no longer occurs. Fluorescence increases in each cycle, proportional to the rate of probe cleavage.

Molecular beacons also contain fluorescent and quenching dyes, but FRET only occurs when the quenching dye is directly adjacent to the fluorescent dye. Molecular beacons are designed to adopt a hairpin structure while free in solution, bringing the fluorescent dye and quencher in close proximity. When a molecular beacon hybridizes to a target, the fluorescent dye and quencher are separated, FRET does not occur, and the fluorescent dye emits light upon irradiation. Unlike TaqMan probes, molecular beacons are designed to remain intact during the amplification reaction, and must rebind to target in every cycle for signal measurement.

Another aspect of the invention relates to a kit for the detection of telomerase activity in a biological sample. In an embodiment, the kit comprises an extension composition containing a first primer and nucleoside triphosphates, and a detection composition containing a second primer, nucleoside triphosphates, a double-stranded DNA binding dye, and a DNA polymerase. The extension composition is capable of producing an extension product from the first primer when mixed with a telomerase; and the detection composition is capable of amplifying the extension product in a PCR reaction and generating a labeled amplification product for quantification.

What is claimed is:

1. A method for detecting and quantifying telomerase activity in a biological sample, said method comprising the steps of:

(a) adding the biological sample to a reaction mixture comprising a first primer and nucleoside triphosphates,

(b) incubating the reaction mixture under conditions suitable for a telomerase to produce an extension product from the first primer; and

(c) quantifying the extension product using real-time PCR analysis.

2. A kit for detecting telomerase activity, said kit comprising:

an extension composition comprising a first primer and nucleoside triphosphates, said extension composition is capable of producing an extension product from the first primer when mixed with a telomerase; and

a detection composition comprising a second primer, nucleoside triphosphates, a double-stranded DNA binding dye, and a DNA polymerase, said detection composition is capable of amplifying the extension product in a PCR reaction and generating a labeled amplification product for quantification.

3. The kit of claim 2, wherein the extension composition and the detection composition are stored in a single container, and are separated by a layer of wax in said container.